Physicochemical analysis, elemental and amino acid composition of wool and silk.

TSH Amirova[1*], AA Ibragimov[1], OM Nazarov[2], OM Nazarov[1]

[1]Department of Chemistry, Fergana State University, Fergana, Uzbekistan

[2]Department of Department of Chemistry, Fergana State University Fergana, Uzbekistan

Abstract

The ash content of wool and silk was determined by burning the corresponding samples. The elemental composition of wool and silk was determined by ICP-mass spectral analysis, 26 macro-and microelements were found. Phosphorus has the maximum content in wool and silk samples, magnesium has the minimum content for wool, and potassium for silk. Chromato-mass spectrometry revealed 17 amino acids in wool and silk samples. Wool is characterized by a high content of phenylalanine, glycine, tyrosine, while silk is characterized by a high content of serine, glycine and threonine. Wool and silk proteins are also balanced in terms of nonessential amino acids. The analysis of the obtained IR spectra of proteins from wool and silk is carried out, absorption bands for the corresponding groups are marked. The corresponding vibrations of the amino groups of the carbonyl group are shown, which have an essential role in the structure of proteins.

Keywords: Wool, Silk, Elemental composition, Amino acids, IR spectra.

Accepted on October 05, 2021

Introduction

Silk is a natural protein fiber, some forms of which can be used in textiles. Silk protein fiber consists mainly of fibroin and is produced by the larvae of some insects to form cocoons. The most famous silk is obtained from the cocoons of silkworm caterpillars (*Bombyx mori Linn*.) and related species. The silkworm or silkworm belongs to the family of silkworms (*Bombycidae*). Silk protein is a type of protein like collagen, elastin, keratin, fibroin, sporgin, and others, and is an indispensable component of the cocoon thread. Silk fiber protein is synthesized by the cells of the silk glands and stored in the lumen of the silk glands. It is subsequently converted into silk fibers. The shimmering appearance of silk is due to the triangular prismatic structure of the silk fiber, which allows the silk fabric to refract the incident light at different angles, thus creating different colors. The raw silk of domesticated silkworms exhibiting a natural luster. Silk has a smooth, soft texture that is not slippery, unlike many synthetic fibers. Silk is one of the strongest natural fibers, but when wet it loses up to 20% of its strength. Has a good moisture recovery of 11%. Silk does not conduct electricity well and is therefore susceptible to static electricity. Silk has a high emissivity of infrared light, which makes it cool to the touch. The silk secreted by the mulberry worm is composed of two main proteins, sericin and fibroin, with fibroin being the structural center of silk and sericin being the sticky material that surrounds it. Fibroin is composed of the amino acids Gly-Ser-Gly-Ala-Gly-Ala and forms beta-pleated sheets. Hydrogen bonds are formed between the chains, and side chains are formed above and below the plane of the hydrogen bond network. The high proportion (50%) of glycine ensures a tight packing. The addition of alanine and serine makes the fibers strong and resistant to tearing. This tensile strength is due to the many interconnected hydrogen bonds, and when stretched, a force is applied to these multiple bonds without breaking. Silk is resistant to most mineral acids, with the exception of sulfuric acid, which dissolves it. Will turn yellow with sweat. Chlorine bleach also damages silk fabrics [1-3].

Wool is a textile fiber obtained from sheep and other animals. Wool is composed of protein and a small percentage of lipids. In this respect, it is chemically very different from the more dominant textile, cotton, which is mainly composed of cellulose. Wool is produced by follicles, which are small cells located in the skin. These follicles are located in the top layer of the skin called the epidermis and are pushed down into the second layer of skin called the dermis as the hair fibers grow. Follicles can be classified as primary or secondary follicles. Primary follicles produce three types of fibers: Kemp, core fibers, and real wool fibers. The secondary follicles only produce true wool fibers. Wool has a high thermal resistivity and therefore generally inhibits heat transfer. Wool can absorb up to a third of its weight in water. Wool absorbs sound like many other fabrics. Wool ignites at a higher temperature than cotton and some synthetic fibers.It has a lower flame propagation rate, lower heat generation rate, lower heat of combustion does not melt or drip; it forms an insulating and self-extinguishing carbonized material that is less conducive to the formation of toxic substances, gases and fumes. Wool and natural silk are animal fibers. Woolen fibers and natural silk are mainly composed of protein. Many works are devoted to the isolation of individual proteins from wool and silk, as well as to the study of their amino acid composition and structure [4-8].

The purpose of our research is physicochemical analysis and determination of the elemental and amino acid composition of wool and silk samples of Uzbekistan.

Materials and Methods

Wool and silk samples provided by Margelan Research Institute of Natural Fibers.

Determination of ash content

The analysis was performed using the recommended method in duplicate. We used an analytical balance, a muffle furnace, and a desiccator. The ash content was determined by burning a sample in a muffle at a temperature of 6000C-80000C, for 2-3 hours, until the presence of organic substances in the ash disappeared in the form of black particles. The ash content was determined by the difference between the crucible mass before and after calcination in the muffle, expressed as a percentage of the initial sample, according to the formula [9-12].

$$Z = \frac{M_1 - M_2}{H} \cdot 100$$

Where, M1 is the mass of the crucible with a sample before drying, gr.

M2 is the mass of the crucible with a sample after drying, gr.

N is the weight of the sample, gr.

Method for determining the mineral composition

In order to determine the elemental composition of the wool and silk object submitted for investigation, ICP-mass spectral analysis was carried out on an ICP-MS instrument (inductively coupled plasma mass spectrometer) AT 7500.

Preparation of the object for analysis

From the object of wool and silk, weighed samples of ash with a mass of 0.1 g were taken into heat-resistant cones. In duplicate, 30 ml of concentrated nitric acid was added and decomposed by boiling on a hotplate for 30 minutes until a clear solution was obtained. Then the resulting solutions were filtered into 100 ml volumetric flasks and made up to the mark with distilled water. The samples prepared in this way were analyzed on an inductively coupled plasma mass spectrometer in the "Semiguant" semi-quantitative analysis mode. Device parameters: plasma power 1200 W, integration time 0.1 second. The instrument was calibrated and quantitatively calculated based on the Agilent Technologist mlti-element calibration standard, 22 elements [13-15].

Protein Quantification Method

Apparatus, materials and reagents

Determination of the protein content in wool and silk tissues was carried out by the standard method. We used laboratory scissors, analytical balance (0.0001), filter paper, conical funnel, FEC, sodium hydroxide, Rochelle salt, Nessler's reagent, distilled water, concentrated sulfuric acid, and concentrated hydrogen peroxide.

Analysis

The study of protein substances is carried out by various methods. However, all methods of studying proteins are reduced to the following. To isolate proteins, biological material is crushed until the cell walls are destroyed, obtaining a homogenate. Then proceed to the extraction of proteins.

To determine the protein content in the isolated fractions, an aliquot of them was taken into a heat-resistant flask (from 5 to 10 ml). Concentrated sulfuric acid H2SO4 (p=1.84 g/cm3) was poured into heat-resistant flasks, to a selected sample or to a taken aliquot of the fraction. The flasks were placed in a sand bath, setting the temperature equal to 400 °C. At the same time, it is necessary to avoid violent boiling. Distilled water was carefully poured into cooled flasks along the walls and quantitatively transferred into a volumetric flask with a capacity of 50 ml. After cooling, the volume in the flasks was brought to the mark and mixed thoroughly. From a volumetric flask, after mineralization, to determine the protein content by nitrogen, an aliquot was taken, depending on the expected protein content. At a high nitrogen content in the samples, dilution was carried out. To the selected aliquot, up to half the volume of distilled water was added. Then the solution was neutralized. And added 1 ml of Nessler's reagent. The solutions in the flasks were brought to the mark with water and mixed thoroughly. In this case, the solutions should be completely transparent. 15 minutes after staining, the solutions were colorimetric on a KFK-3 electrophotocolorimeter [16-18].

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Protein Isolation M*ethod*

Extraction

Extraction was carried out on a magnetic stirrer with 0.2 N sodium hydroxide in a ratio of 1:10. Cell debris was removed by centrifugation in a RS-6 refrigerated centrifuge within 30 minutes at 6000 rpm. The resulting clear supernatant (supernatant solution) was precipitated with ammonium sulfate with stirring on a magnetic stirrer. The resulting extract was left in the refrigerator for 16 hours to form the protein. Then the extract was centrifuged at 6000 rpm for 30 minutes in a refrigerated centrifuge. The resulting precipitate was collected and dissolved in a minimum volume of 0.2 N sodium hydroxide. Dialysis of the obtained protein solutions. Dialysis (desalting) of the obtained protein solutions was carried out in running water for 24 hours in cellophane bags in glass containers. The property of cellophane bags for dialysis differs in that the cellophane bags soaked in water for 2 hours have the property of passing substances with a molecular weight of less than 10,000 Dal [22-24].

Freeze drying of protein solutions

The protein solutions desalted after dialysis were lyophilized at a temperature of -35°C and a high vacuum created by a vacuum pump. In a colorocryostat (a freezer containing ethyl alcohol cooled to -35°C) in round-bottom flasks (0.5 ml) with thin section No. 29, protein solutions were frozen in an even layer, then placed on freeze-drying. Drying takes place within 6-8 hours.

HPLC analysis of FTK-derivatives of amino acids

The synthesis of FTC (phenylthiocarbomail) derivatives of free amino acids was carried out according to the method of Steven et al. The identification of FTC amino acids is carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 mm Discovery HS C18 column. Solution A: 0.14 M CH3COONa +0.05% TEA pH 6.4, B: CH3CN. Flow rate 1.2 ml/min, absorption 269 nm. Gradient% B/min: 1%-6%/0-2.5 min; 6%-30% / 2.51-40 min; 30%-60%/40.1-45 min; 60%-60%/ 45.1-50 min; 60%-0%/50.1-55 min [25,26].

Results and Discussion

The ash content of wool and silk was determined by heating the samples at a high temperature, while the ash content in the wool sample is much higher than that in silk (Table 1). The total protein content was determined according to the classical method. In silk, the protein content is not much higher than in wool (Table 1). The nitrogen content was calculated based on the average nitrogen content in proteins (16%, 100:16=6.25).

No.	Name	Ash content %	Nitrogen %	Protein %
1	Wool	2,88	13,0	81,8
2	Silk	0,75	13,2	82,5

Table 1. *Results of physical and chemical analysis of wool and silk.*

In wool and silk samples, 26 macro and microelements were found. Table 1 shows data on the content of macro and microelements in wool and silk samples. The results of the analysis are shown in Table 2.

Name of elements	Content of main and impurity elements, g/kg	
	Wool	Silk
Na	1,83	1,45
Mg	0,67	0,35
Al	0,081	0,073
P	3,79	3,35
S	1,308	0,195
K	1,007	0,187
Ca	1,439	1,043
Ti	0,017	0,022
V	0,00046	0,00049
Cr	0,0209	0,0256
Mn	0,0096	0,0055

Fe	0,484	0,512
Co	0,00038	0,00036
Ni	0,0109	0,0138
Cu	0,0327	0,0276
Zn	0,0379	0,0096
As	0,0026	0,0011
Se	0,0432	0,0590
Mo	0,0029	0,0028
Sn	0,379	0,216
Sb	0,00026	0,00043
I	0,0065	0,0092
Ba	0,0157	0,00787
Hg	0,000929	0,001142
Pb	0,0013	0,0019
Bi	0,0023	0,0003

Table 2. *Mineral composition of the presented samples, g/kg.*

The following macroelements were found in wool and silk samples: Na, Mg, P, S, K, Ca (Figure 1).

Decreasing order of macronutrient content for wool P>Na>Ca>S>K>Mg, for silk P>Na>Ca>Mg>S>K. Wool has a higher macronutrient content than silk, *i.e.* 10.044 and 6.575 g/kg, respectively.

The highest content in wool and silk samples for P, the values are 3.79 g/kg and 3.35kg/kg, and the lowest content for wool Mg (0.67 g/kg) and silk K (0.187 g/kg) (Table 1).

In the samples of wool and silk, 17 trace elements were found (Figure 2).

The iron content was the highest among trace elements, for wool (0.484 g/kg) and silk (0.512 g/kg). Vanadium, cobalt and antimony have the lowest content among microelements (Table 2).

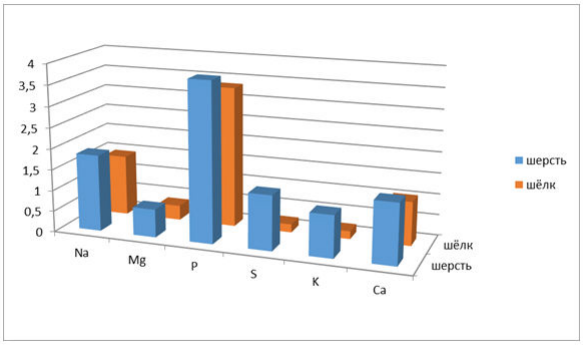

Figure 1. *Macronutrients found in wool and silk samples.*

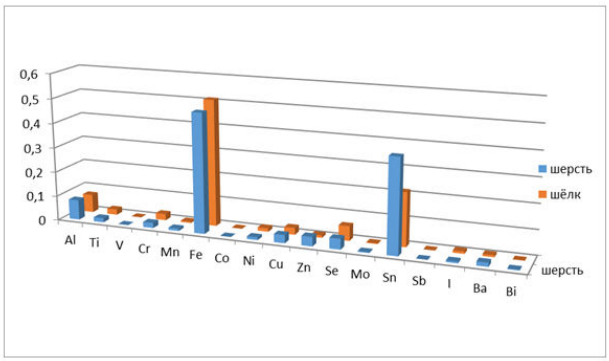

Figure 2. *Trace elements found in wool and silk samples.*

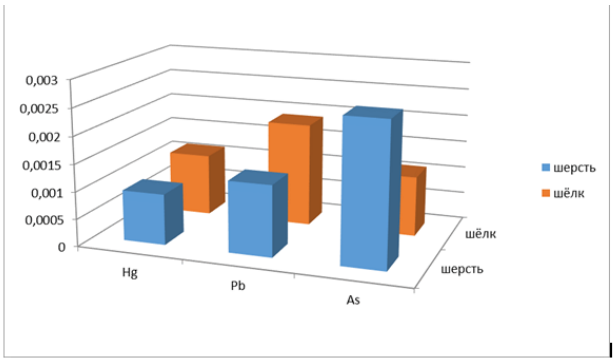

Figure 3. *Toxic elements found in wool and silk samples.*

Among toxic elements, mercury, lead and arsenic were found (Figure 3). Their content is significantly less than the MPC for food products.

The content of amino acids in wool and silk samples was also investigated, the results are shown in Table 3. 17 amino acids were found in wool and silk samples. Asparagine, glutamine and tryptophan were not detected in both samples. The total

amount of amino acids in silk is 96.70825 mg/g. The highest contents in wool are phenylalanine (19.31486 mg/g), glycine (16.00736 mg/g) and tyrosine (10.93829 mg/g).

The lowest content in wool has lysine (1.584618 mg/g) and proline (0.722736 mg/g). The total amount of amino acids in silk is 413.5124 mg/g, which is much higher than in wool.

The highest contents in silk are found for serine (105.6573 mg/g), glycine (72.85463 mg/g) and threonine (32.72677 mg/g). It is noteworthy that the amount of serine is greater than the total amount of amino acids in the wool.

Proline (3.378736 mg/g) and histidine (3.631299 mg/g) have the lowest contents in silk.

The following essential amino acids are found in wool and silk: threonine, methionine, isoleucine, valine, phenylalanine, leucine and lysine.

The total content of irreplaceable amino acids in wool is ~39 mg/g (37.7%), and in silk ~120 mg/g (29%).

An aromatic hydrophobic amino acid containing the indole nucleus tryptophan was not found in silk and wool samples. Wool and silk proteins are also balanced in terms of nonessential amino acids.

The content of hydroxyl-containing amino acids in silk (40.5%) is much higher than in wool (22%). Judging by the sum of amino acids, silk proteins are much higher in quality (Figure 4).

Amino acids	Wool	Silk
	Concentration mg/g	
Asparagine acids	19,13,927	26,22,601
Glutamine acids	53,33,787	16,97,671
Serin	70,55,695	10,56,573
Glycine	16,00,736	72,85,463
Asparagine	0	0
Glutamine	0	0
Cysteine	29,00,615	66,61,144
Threonine*	32,65,892	32,72,677
Argenine	22,45,763	2,16,813
Alanine	61,66,583	7,43,656
Proline	0,722736	33,78,736
Tyrosine	10,93,829	29,23,522
Valine*	31,42,891	16,66,065
Methionine*	31,67,436	80,11,786
Isoleucine*	20,69,429	64,92,401
Leucine*	64,50,528	97,61,661
Histidine	44,27,843	36,31,299
Tryptophan* {{ 1}} Phenylalanine*	0	0
Lysine HCl*	19,31,486	27,42,364
Total	15,84,618	18,69,656
Proline	96,70,825	41,35,124

Table 3. *Amino acid composition of wool and silk proteins.*

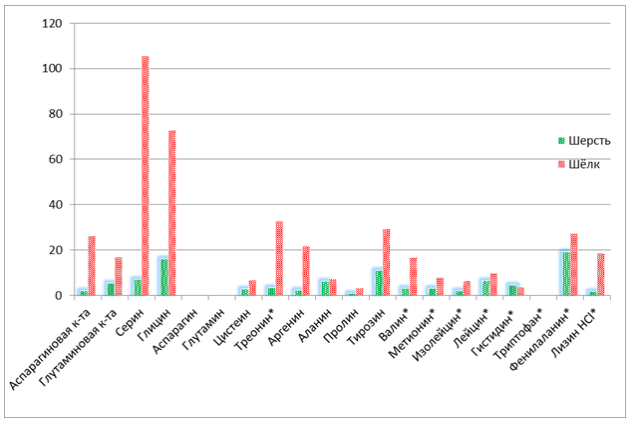

Figure 4. *Comparison of amino acid content in wool and silk samples.*

When analyzing the obtained IR spectra of proteins from wool and silk, it was noted that absorption bands at 3500–3000 cm-1 are associated with the stretching vibration of N-H. The O-H stretch band is located at 3600-3200 cm-1, which overlaps with the peak of the N-H stretching vibration at 3500-3000 cm-1 (for wool, the peak at 3423.59 and for silk, the peak at 3373.55 cm-1) (Figure 5 and 6). Absorption bands at 1656.02 cm-1 (Amide 1 band) for silk and 1569.25 cm-1 for wool due to deformation vibrations of aminocarbonyl (CO-NH) groups of the peptide bond. As well as a decrease in the intensity of absorption bands at 1532.64 in the case of silk and 1418.23 cm-1 in the case of wool due to deformation vibrations of amino groups (-NH_2) and stretching vibrations of the carbonyl group (-CO) (band Amide 2).

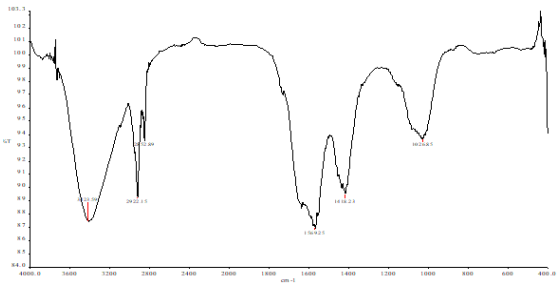

Figure 5. *IR - spectrum of wool.*

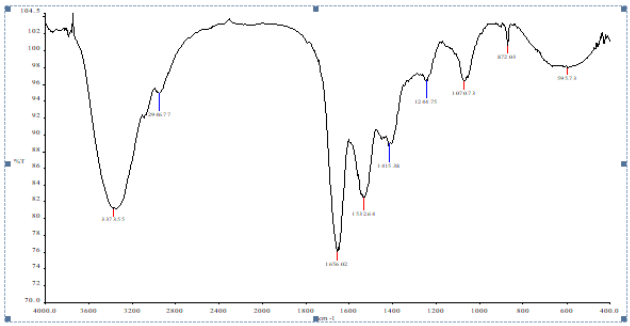

Figure 6. *IR spectrum of silk.*

Conclusion

When analyzing the obtained IR spectra of proteins from wool and silk, it was noted that absorption bands at 3500–3000 cm-1 are associated with the stretching vibration of N-H. The O-H stretch band is located at 3600-3200 cm-1, which overlaps with the peak of the N-H stretching vibration at 3500-3000 cm-1 (for wool, the peak at 3423.59 and for silk, the peak at 3373.55 cm-1). Absorption bands at 1656.02 cm-1 (Amide 1 band) for silk and 1569.25 cm-1 for wool due to deformation vibrations of aminocarbonyl (CO-NH) groups of the peptide bond. As well as a decrease in the intensity of absorption bands at 1532.64 in the case of silk and 1418.23 cm-1 in the case of wool due to deformation vibrations of amino groups (-NH_2) and stretching vibrations of the carbonyl group (-CO) (band Amide 2).

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*Corresponding to:

TSH Amirova

Department of Chemistry

Fergana State University

Uzbekistan

Tel: 998905344333

E-mail: egulamov93@gmail.com